The genetics of type II diabetes and genetically modified organism technology for insulin

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Diabetes is one of the fastest growing health risks throughout the world, and the most notable approach to exploring the disease has been the study of different genes and environmental factors that affect susceptibility to type I and type II diabetes (T2D). Behind every diagnosis is an intricate web of differences that lead to the inability to process glucose for energy as a result of insulin resistance and insufficient insulin production. Even with the common symptoms of high blood glucose levels and metabolic dysfunction, certain populations have been found to carry higher risks for developing the disease than others. This raises the questions of how and why genes and environment can have differing effects on particular groups. Scientists turn to genetic linkage studies in order to answer these questions. Obesity research and genetic profiling are important contributors to the progress in diabetes education, and genetic modification has made the development of treatments possible despite the largely unknown genetic contributions to T2D. Type II diabetes is a complex, multifactorial disease that differs in genetic markers among populations including African-Americans, Japanese, and women, but genetic modification technology offers artificial insulin production for universal management of T2D despite its complexity.

One diagnosis of diabetes is not always comparable to another; rather, diabetes is a spectrum of combined causes and symptoms that exist between strictly type I and strictly type II in all populations. Type II diabetes is a result of metabolic dysfunction instead of the autoimmunity against functional insulin production that is characteristic of type I. The variance in the causes shows correlation with genetic and environmental factors. Because these are fundamentally different modes of developing diabetes, the underlying genetic variants are a major source of research in the effort to prevent and treat T2D. Genome wide association studies (GWAS) have been successful in determining the relevance and significance of over 500,000

single nucleotide polymorphisms (SNPs); these analyses are just some of many that are important in distinguishing patterns of disease development, as each study analyzes different populations that exhibit symptoms of T2D. Single nucleotide polymorphisms in the calpain-like protease (*CAPN10*) and transcription factor 7-like 2 (*TCF7L2*) genes that are related to T2D are some of the first SNPs identified through linkage studies. *CAPN10* encodes for calpain 10, which is a cysteine protease involved in glucose metabolism, and *TCF7L2* encodes for a protein that acts like a transcription factor (Groop & Pociot, 2013). While the mechanisms and effects of these genes are not clear, the genomic analysis promotes deeper study to more finely map genomes, especially at these loci, in all populations. Different SNPs in the analysis of another gene at the B-cell lymphoma 2 (*BCL2*) locus encoding for an anti-apoptosis protein were also found to increase the T2D risk in not only African-American populations, but also Asian and Hispanic groups (Saxena *et al.*, 2012). Single nucleotide polymorphisms at these loci do not always identify the genes associated with diabetes, but they are the main variant markers in genomes that are studied to understand the genetic basis of diabetes between separate populations.

Genetic determinants for diabetes analyzed in genomic studies have shown to be more different than similar across different populations of people. Palmer *et al.* (2012) performed a GWAS where the rs7560163 SNP in the Rho family GTPase 3 (*RND3*) gene that encodes for Rho family GTPase 3 showed a stronger, more direct correlation to T2D in African-American populations than in European populations. In previous GWAS, the rs7560163 SNP had not reached genome-wide significance, but for the meta-analysis of 6,449 African-American individuals in this study, the SNP was found significant ($P= 7.0 \times 10^{-9}$). The major allele for diabetic tendencies was also found to be more prevalent in representative African samples from

Nigeria, suggesting that the diabetic traits might be under selection in these populations and that the African-derived allele is more deleterious in western culture (Palmer *et al.,* 2012). These findings suggest a higher risk for diabetes among African-American populations that could be explained by polymorphisms that are not equally present in other groups, like those of European descent. The SNPs that are distinct for a certain group are important indicators of evolutionary selective pressures that may come from descent or culture which result in one population with a higher rate of disease. These African-derived alleles, for example, give African-Americans a higher risk of T2D compared to those with the European allele in the same environment due to the interaction possibly between the diet and culture in the environment and the difference in allele type. This one SNP governs important differences between groups of European and African descent, but how it correlates with other polymorphisms within that gene or other parts of the genome related to T2D would also be important to know to fully understand differences in disease development.

Even within a given population, the presence or absence of SNPs can alter disease risk, further complicating the study of diabetes. A study by Fujita *et al.* (2012) found that including more SNPs associated with disease risk in analysis showed more genetic determination of T2D in Japanese populations, suggesting that the disease risk SNPs have an additive nature. The SNP of fatty acid desaturase 1 (*FADS1*) that encodes for a fatty acid desaturase and another near Prospero Homeobox I (*PROXI*) that encodes for a homeobox transcription factor also showed association with risk of T2D, which is the first time this correlation has been observed (Fujita *et al.,* 2012). This study analyzed nine different SNPs that were associated with T2D (P=0.0078) in a sample of 2632 Japanese participants with T2D and 2050 participants without. The participants who possessed more disease-risk SNPs exhibited a higher fasting plasma glucose levels and a

higher risk of T2D (P=0.0017). The additive nature of these SNPs is not the same for the SNPs in African-American populations and may even differ between Japanese populations, but it is important to note that observing other minor SNPs in different groups may increase the genetic correlation between T2D and a certain genotype.

On top of the array of genetic determination, the added component of interaction between environmental influences and these genetic dispositions in distinct groups increases the complexity of disease analysis. An important environmental factor associated with T2D is diet and, subsequently, body mass index (BMI). Body mass index can be influenced by genetic predispositions like metabolism, but diet and exercise are major determinants that can correlate BMI with diabetes. A BMI of greater than 30 kg/m^2 is considered obese, and a diet high in carbohydrates and fats can raise BMI over time without the mediating effects of exercise. Most individuals with T2D are also obese and have been shown to also carry the obesity-linked *FTO* gene that encodes for the alpha-ketoglutarate-dependent dioxygenase enzyme (Dimas *et al*., 2014); this gene is thought to control energy usage and intake. Because it is well established that obesity is correlated with the risk of T2D, the effect of body mass index (BMI) was included into the study by Palmer *et al*. (2012), and women were found to be another distinct population at risk for T2D. Factoring BMI into the GWAS of different loci previously known to be associated with diabetes showed that seven of the ten associated disease risk loci were more prevalent in women (Palmer *et al.,* 2012). This is a much larger population than the previous groups of different ethnicities, which makes it difficult to draw direct conclusions about determinants of disease risk. However, accounting for BMI introduces a factor that is partially environmental and partially genetic. BMI can be influenced by diet, exercise, and other environmental factors like culture to a certain degree. Women will experience different selective pressures based on their

reproductive roles, creating a disparity between men and women evolution of BMI related characteristics and subsequently risk of T2D. The correlation of BMI and disease risk exemplifies that groups of people can be differentiated based on environment and evolutionary pressures resulting in selection, and that proposes more possible influences outside of genetic markers that affect expression of disease.

The numerous SNPs found to be associated with disease risk for T2D in different populations need selective attention to resolve the exact genetic correlation in each population, which makes it more difficult to form a universal cure. Sometimes these polymorphisms and alleles are rare, but may contribute through different interactions with the genome and the environment. Even with the analysis of SNPs at many genes, there lies the possibility that some mutations at certain loci have been overlooked or have not been found yet. Some of the mutations may even have a more negligible effect than predicted due to unanalyzed interactions with other factors (Fujita *et al.,* 2012). All of these combined possibilities alongside factors like BMI that can be affected by environment and evolutionary pressures leave it difficult to treat every diagnosis because there is still much to be discovered in order to understand the physiology of T2D. The starting point for devising the closest thing to a universal cure is hormones involved in metabolism and energy, like insulin, because most of the genes associated with T2D have some role in those pathways.

Even with the similarity of metabolic disruption, the amount of variation in diabetes makes it difficult to treat and control because the genetic and environmental factors vary in strength and severity among all individuals. Insulin is the main target for treatment because it is one of the factors in the symptoms that is common among diabetics. Insulin is the key component in metabolizing glucose as a source of energy; it is a hormone produced by the

pancreas that signals for cells to take up glucose from the bloodstream and break it down or store it as energy. Type II diabetics generally have insulin resistance where cells cannot respond to normal levels of insulin and therefore cannot take in glucose, resulting in high blood glucose levels which can ultimately lead to the need for higher levels of insulin than the body can produce. Insulin injections are then used to accommodate the extra insulin needed break down glucose. This is the most common treatment available and it has developed significantly over the last few decades. Although insulin injections are one of the most common forms of treatment for diabetes, the production of this insulin is costly and does not always work effectively for every diabetic. Previous methods of acquiring insulin for treatment involved extracting the insulin produced by other animals such as pigs, which was very expensive and often elicited allergic reactions. The solution to this was genetic modification of *E. coli* to possess the human insulin gene, which is effective in producing large amounts of insulin that is less expensive and safer to use. Genetic modification involves the insertion of a foreign gene into a new genome usually through plasmids in bacteria, and genetically modified organisms (GMOs) are now the main method of insulin production. In a study done by Boyhan *et al.* (2011), the DNA in tobacco and lettuce chloroplasts was altered to include the modified plasmid vector with the human insulin production and *CTB-PFx3* genes to produce proinsulin with cholera toxin B (CTB). The CTB fusion protein helps protect the insulin during the purification process and augments its uptake inside the body (Boyhan & Daniell, 2011). These tobacco and lettuce plants aim to make insulin even more accessible and are an even cheaper way to produce larger quantities of insulin that would be more effective as a treatment for some diabetics. Until genetic modification can fix the autoimmunity and metabolic dysfunctions, new genetically modified organisms like these tobacco and lettuce plants as well as the common organisms already in use like *E. coli* are the

main source of insulin producing technology to alleviate diabetic stress. As more studies reveal genetic linkage in T2D, gene therapy and individualized treatments may be used to increase effectiveness of treatment.

Research through GWAS and linkage studies on the causes and cures for T2D is quickly developing and integrating the study of genetic markers for disease risk usually in the form of SNPs. Even though these genome association studies show strong correlations between certain polymorphisms and diabetes, the range of alleles and SNPs is very broad among distinct populations. For example, the rs7560163 SNP in African-American populations and the SNPs of *FADS1* and *PROXI* in Japanese populations are distinct markers from SNPs in European alleles and populations. The differences in these lead to alterations in disease risk that may be associated with evolutionary selective pressures at these loci. These genetic factors and selective pressures can also be influenced by environment, and that manifests itself in things such as BMI, which distinguishes women from men. Women are at a higher risk for T2D because of the female body's ability to retain fat for reproductive periods; this results in a higher BMI which is also correlated to risk of T2D specifically in obese individuals. This same effect, however, can be achieved in other individuals or groups with high carbohydrate and fat diets, which are likely to be a product of environment, culture, or lifestyle. These interactions make it even more difficult to distinguish causality in diabetes and to devise a cure, but the GMO market is promising in the field of cheaper and more effective insulin production to treat diabetes in all populations, regardless of ethnicity or gender. With the advancement of gene modifying techniques, researchers have an increasing number of options, like tobacco and lettuce leaves, to refine insulin production, making insulin easier to access and cheaper to produce. GMOs enable more

diabetic populations to access insulin and are the best option for treatment until more of the genetic factors and interaction with environment can be understood.

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